(NEW SERIES) No. 51

SCIENTIFIC MEMOIRS

BY

OFFICERS OF THE MEDICAL AND SANITARY DEPARTMENTS

OF THE

GOVERNMENT OF INDIA

A STREPTOTHRIX ISOLATED FROM THE SPLEEN OF A LEPER

BY

MAJOR W. G. LISTON, M.D., D.P.H., I.M.S.,

AND

CAPTAIN T. S. B. WILLIAMS, I.M.S.

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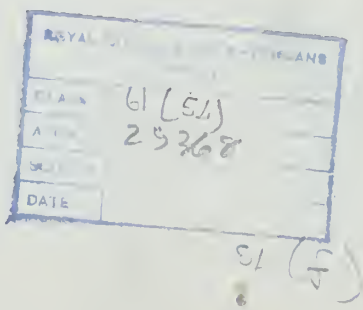

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A Streptothrix Isolated from the Spleen of a Leper.

IN the following paper we propose to record the results of our observations on a pure culture of a streptothrix which we isolated from the spleen of a leper. The case is of interest for two reasons, firstly, because the streptothrix closely resembled that described by Deycke Pasha[1] and Major Rost,[2] and secondly, because of the curious variations of the growth in respect to (*a*) morphology, (*b*) staining reactions, and (*c*) pigment production.

The post-mortem examination of this patient was made $1\frac{1}{2}$ hours after death. The liver and spleen were much enlarged and showed vast numbers of acidfast " lepra bacilli " in smears made from them.

The spleen in particular was more carefully examined and removed to the laboratory for the purpose of making cultures and sections. In all, some thirty-nine tubes of various media were smeared with the splenic pulp carefully removed with all aseptic precautions from different parts of the spleen. The media used comprised tubes of ordinary peptone agar, lemco-agar, " nasgar," blood serum, blood agar, Dorset's egg medium and a cheese medium. To some of the tubes a pure mixed culture of an amœba was added, while bacteria free amœba cysts were placed on other tubes. The majority of the tubes, however, were simply smeared with splenic pulp. In each of four of the thirty-nine tubes a single colony of a golden yellow colour developed in the course of three or four days. All the tubes remained sterile except those to which a pure culture of a bacillus had been added together with amœba cysts and the abovementioned four tubes in each of which a single yellow colony developed. The four yellow colonies resembled each other closely, and on further study were shown to belong to one species of streptothrix. One of the colonies, namely, the one which grew on " lemco-agar," was selected for minute study. The other colonies were found, two in two tubes of Dorset's egg medium alone, and one in a tube of Dorset's egg medium to which a pure mixed culture of amœbæ had been added to the smeared spleen pulp.

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[1] Deutsche Mediz. Wochenschr. Nos. 13 and 14, 1905.

[2] Scientific Memoir by Officers of the Medical and Sanitary Departments of the Government of India, No. 42.

Detailed study of the colony on lemco-agar.

In all the tubes a considerable quantity (a large loopful) of splenic pulp was spread over the surface. The tubes were carefully examined from day to day. On the third day, on the lemco-agar tube, a single minute golden yellow coloured colony was observed. A smear was made from this colony which was stained with carbol fuchsin, decolourised by 25 per cent. H_2So_4 for one minute,* and counter stained with Methylene blue. Illustrations III and IV are drawn from two selected fields of this film. It will be seen that we were dealing with an acidfast streptothrix, which in some parts was losing its acidfast properties.

Twenty-four hours later the colony was again examined. It had increased considerably in size, partly because, in removing some of the culture to make the specimen described above, the growth had been spread over a large surface and partly because the colony itself had grown larger. Smears were again made and stained as above with the result shown in Figure V. It will be noticed that the acidfast portions of the streptothrix were now scarcer, and that the filaments were divided into smaller fragments, resembling bacillary filaments rather than streptothrix filaments. The colony at this time was further spread over the surface of the agar, and, when examined 48 hours later, or six days after the tube had first been planted, showed a thick raised opaque golden yellow mass, as shown in Figure XI. Films made at this stage and stained by the Zeihl-Neelson method showed that the filaments had still further broken up and now resembled cocco bacilli, a few of them non-acidfast but the majority acidfast (Figure VI). At this stage subcultures were made on a number of other media, *viz.*, ordinary agar, cheese medium, Dorset's egg medium, and Rost's medium. The cultures in all these media now grew rapidly so that in twenty-four hours an extensive growth was obtained. The colour of the growth differed with the medium used, thus :—

(*a*) The original growth was golden yellow in colour (Figure XI).

(*b*) A subculture upon ordinary nutrient agar gave a brick-red growth (Figure XII).

(*c*) A subculture from (*b*) upon cheese medium was scarlet in colour (Figure XIV).

(*d*) A subculture from the scarlet growth on cheese medium upon Dorset's egg medium became golden yellow (Figure XIII).

(*e*) A subculture from the original golden yellow colony on lemco-agar upon Dorset's egg medium gave a golden yellow growth.

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* All the slides stained by the Ziehl-Neelson method were treated in this way.

(*f*) All subcultures from Rost's medium when put on Dorest's egg medium gave a golden yellow growth.

A microscopical examination showed that the growth on ordinary agar medium was non-acidfast (Figure VIII) ; on cheese medium and Dorset's egg medium portions were non-acidfast (Figure IX). In old cultures there was a tendency to an increase of the acidfast portions. The individual elements of the growths varied much in shape, from cocal forms (Figure VI), through bacillary forms (Figure VIII), to streptothrix filaments (Figures III, IV and VII). In Rost's medium, however, the growth was for the most part acidfast, and in this medium the streptothrix nature of the growth was more evident (Figure VII). The acidfast property was always developed in this medium although the growth from which the subculture was made was not acidfast. For example, a subculture from an ordinary agar tube, which was not acidfast (Figure VIII), was put into Rost's medium. A specimen prepared from this medium after twenty-four hours was acidfast and the streptothrix nature of the growth was more evident (Figure VII).

Meanwhile, sections of the spleen which had been hardened and dehydrated in alcohol and imbedded in paraffin with chloroform were cut and examined by Captain Taylor, I.M.S. The sections were stained by various methods. It was found that the imbedding process had made the "lepra bacilli" practically non-acidfast so that Gram's method of staining was found to give the best results. In one portion of the spleen, which had been treated and stained in this way, in addition to clumps of ordinary endocellular "lepra bacilli" filaments of streptothrix were found apparently growing in a blood sinus, and extending into the adjacent splenic tissue. Sections made from several other portions of the spleen failed to show the streptothrix; a portion of this Gram positive streptothrix growth has been illustrated in Figure I, and is probably the same acidfast streptothrix which was isolated in culture.

The details we have given above showed that we were dealing with a very pleomorphic organism, *i.e.*, a streptothrix which under certain circumstances not only varied greatly, (*a*) in shape, resembling at one time a coccus, at another time a bacillus, and yet a third time showing typical long streptothrix filaments, but (*b*) varied greatly in its acidfast properties, being at one time completely non-acidfast, and at another time entirely acidfast, the acidfastness depending on the age of the growth, and on the nature of the culture medium, and (*c*) varied slightly in the character of the pigment produced in different media. These pleomorphic features of our streptothrix are recognised to be common to other streptotriceæ, a feature of these growths which has been ably pointed out and illustrated by Foulerton, in his Milroy lectures for 1910.

We take this opportunity of drawing attention to the fact, that much useful work may be rejected if the pleomorphic qualities of the streptotricæ are not kept in mind. Had we, for example, by chance not examined the minute colony which developed on the third day in our lemco-agar tube till the colony had grown larger, as on the fourth day, we would have found such appearances as are illustrated in Figure V, and we would not have been regarded as very foolish if we had rejected our cultures as possible contaminations, for there does not appear to be any connection between the acidfast lepra bacilli and the non-acidfast bacillary bodies shown in the figure. However, we are not yet prepared to state, till after further study, that there is any connection between this particular streptothrix which we have isolated and the lepra bacilli found within certain cells in the tissues. We may, however, put forward the suggestion that the causative organism of leprosy may grow in the form of a streptothrix in various parts of the body—in a blood sinus of the spleen, for example, as we found in our case :—growing thus the streptothrix may break up into bacillary filaments and these filaments may be scattered by the blood stream and be taken up by endothelial cells and other macrophages where they would appear as the so-called "lepra bacilli" within the "lepra cells." We can conceive that the majority of these "lepra bacilli" are dead, and that cultures can only be obtained from a leprosy case when living filaments or spores are encountered, perhaps only with great difficulty and after much search in some remote part of the body.

A further point of interest is the following. Previous to the colony on the original lemco tube being spread widely we examined films made from portions of the smeared material which showed no obvious growth. Our object was to see if the "lepra bacilli" in those portions showed any changes which might connect them with the acidfast streptothrix growing in another portion of the same tube. Very interesting changes were observed. See Figure II. In the first place only acidfast organisms were seen, and these varied in shape from typical "lepra bacilli," to large fat bacilli, and even comparatively long acidfast filaments. The illustration is not taken from a single field, but individual elements showing the various changes described above were selected from several fields and grouped together as seen in the illustration.

There were present :—

(*a*) Typical "dotted" lepra bacilli ;

(*b*) Lepra bacilli where the "dots" were becoming much larger than normal ;

(*c*) Large swollen globular acidfast bodies ;

(*d*) Parts of acidfast filaments.

By a careful examination of the film one could make out intermediate forms between every stage, from the small thin "lepra bacillus" to the large swollen acidfast filaments mentioned above. Thus individual bacilli were seen where only one "dot" had swollen to a large size leaving the other "dots" normal; while other "bacilli" again were noted with a large terminal acidfast globular swelling. These latter, separated from the remnants of the "bacilli," formed the large acidfast bodies mentioned under (*c*) above. We are not prepared at present to express any opinion on these changes, but prefer to leave the observed facts on record.

Finally, this organism, if inoculated into guinea-pigs may produce at the site of inoculation a nodular swelling. Smears from this show that there are many acidfast cocco bacilli present, nearly all arranged intracellularly, Figure X.

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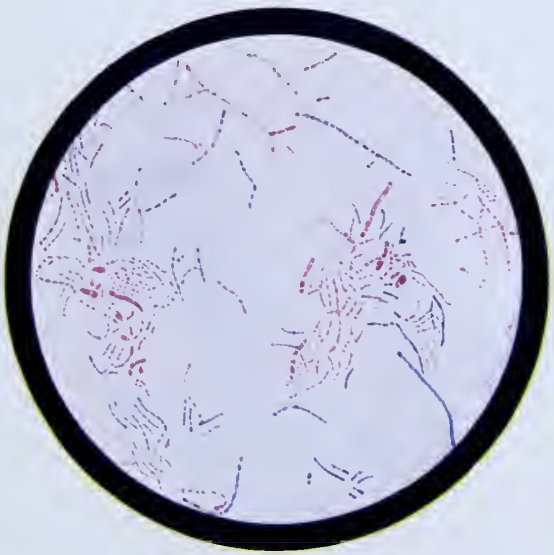

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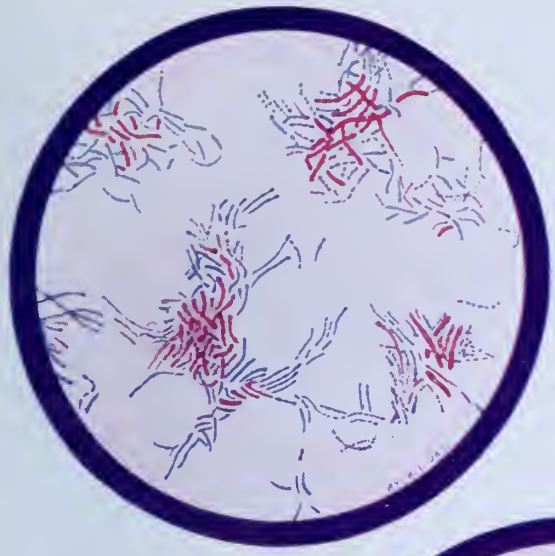

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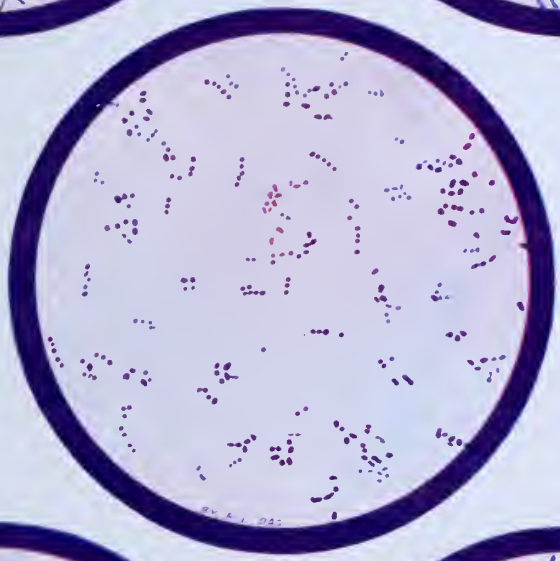

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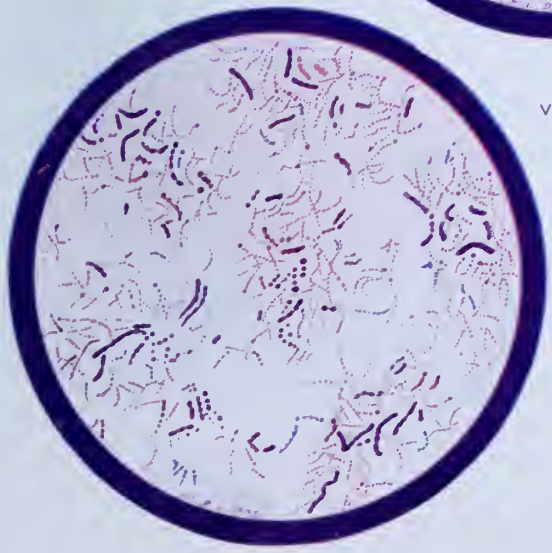

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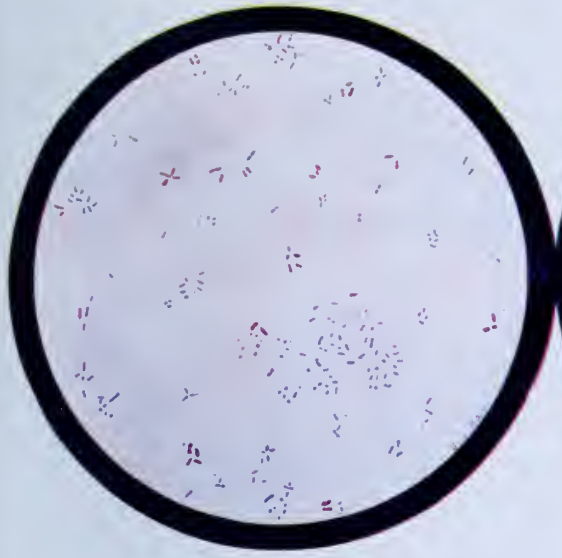

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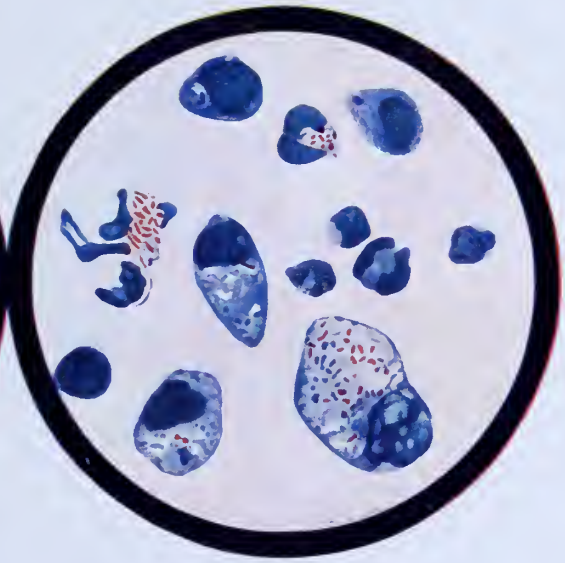

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